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Evaluation of Genotoxic and Cytotoxic Potentials of Uscharin in Male Albino Mice

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Abstract: Uscharin is a new glycoside terpenoid compound extracted from the latex of *Calotropis procera.* Latex-extract has proved to have several therapeutic effects and has been used as insecticide and moluscicide. This study aimed to evaluate Uscharin genotoxicity and cytotoxicity in bone marrow cells of SWR/J albino mice. Uscharin was administered orally to six groups of male mice at three dose levels (1.25, 2.5 or 5 mg /kg body weight) for two time durations (24 and 48h).Uscharin caused significant increase in the number of cells with structural aberrations over the control. The increase was dose-dependent. The most frequent types of structural aberrations were centromeric attenuation and chromosome breakage. The number of cells with numerical aberrations (mainly polyploidy) increased significantly compared to control. The high incidence of polyploidy indicates the carcinogenic potential of Uscharin. It also caused a significant dose-related decrease in the mitotic activity indicating its cytotoxic potential. In conclusion, Uscharin may have a genotoxic and cytotoxic potentials which is demonstrated by the significant increases in chromosomal aberrations and the decrease in mitotic activity.

Key words: Mice Bone Marrow · Uscharin · Chromosomal Aberration · Mitotic Index

INTRODUCTION

The extracts of medicinal plants have formed the basis of many applications, including insecticides, raw and processed food preservatives, pharmaceuticals, alternative medicine and natural therapies [1, 2].

Uscharin is part of the latex-extract of *Calotropis procera*, a giant milk weed, described as a golden gift for human kind where it is used in many therapeutic applications such as antimicrobial activity, anti-inflammatory effects mainly by inhibiting histamine and bradykinin (BK) and partly by inhibiting prostaglandin E2 (PGE2),anti-diarrhoeal activity and antihelmintic activity as reviewed by Sharma *et al.* [3]. The latex-extract contains, in addition to Uscharin, several other compounds such as caoutchouc, calotropin, calotoxin, calactin, trypsin, voruscharin, uzarigenin, syriogenin and proceroside [4]. Latex was reported to possess biological activity against the coccidian protozoa *Eimeria ovinoidalis* [5], *Aedes aegypti* larvae [6] and hyperglycemia induced by alloxan [7] and also it has anti-inflammatory effects [8].The toxic crude latex has been in focus of scientific research in different applications wherein its angiotensin-converting enzyme (ACE) inhibitors activity is reported in *Wistar albino rats* [9] and its antihelmintic activity was evaluated in adult earthworms [10]. The latex-extract of *C. procera* caused a substantial reduction in mitotic index (MI) and induced various types of mitotic and meiotic abnormalities in *Vicia faba* L., which were dose and time-dependent [11]. The cytotoxic and anti-mitotic activities were also observed on the growth of *Allium cepa* roots in a dose-dependent manner [12] and in mice bearing Sarcoma 180 tumor [13].The latex-extract was reported to induce extensive cell death and DNA fragmentation in both Huh-7 (hepatoma cells) and COS-1 (Non-hepatoma cells) [14, 15].

Uscharin, an active glycoside terpenoid compound, was isolated from the latex of *C. procera* which grow wild in eastern desert of Egypt [16]. Uscharin is highly toxic. It is used for inhibiting the activity of the land snails *Thepa pisana* [16, 17]. The aim of the present study was to investigate its possible genotoxic and cytotoxic potentials on SWR/J mice bone marrow cells.

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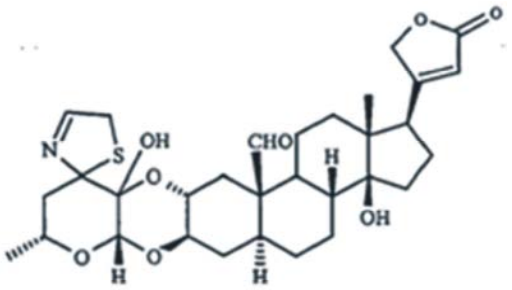
MATERIALS AND METHODS

Animals: Inbred SWR/J albino mice, *Mus musculus* 10-12 weeks old weighing 30-35 g were used in this study. Animals were kept and bred in an environmentally controlled room at a temperature of 22± 1°C, a relative humidity of 45 ±5% and a constant light/dark cycle of 10/14 h. Food and water were given *ad libitum*.

Compound: Uscharin:14-Hydroxy-2,3-((6-hydroxy-9-methyl-8-oxa-1-thia-4-azaspiro(4.5)dec-3-ene-6,7-diyl)bis(oxy))-19-oxocard-20(22)-enolide (2alpha (5S,6R,7S,9R), 3beta, 5alpha)-

As a new substance, uscharin, a colorless, crystallized glucoside which contains sulfur and nitrogen and decomposes at 265C extracted from the latex of *C. procera*.

The compound has the following structural formula:



Uscharin

Dosage and Treatment: Uscharin, dissolved in saline, was administered orally to six groups of male mice (6/group) at three doses(1.25, 2.5 or 5 mg /kg) for two time durations (24 and 48h). The doses correspond to 12.5%, 25% or 50% of the medium lethal dose (LD_{50}), respectively. Uscharin LD_{50} (10 mg /kg) has been determined experimentally. A group of 12 mice were given 0.4 ml of the saline solution each, acted as a control.

Slide Preparation and Analysis: Two hours before sacrifice, the mice were injected intraperitoneally with colchicine (0.6 mg /kg). They were sacrificed by cervical dislocation 24and 48h following treatment with Uscharin or saline. Bone marrow cells, from femurs of treated and control mice, were collected on saline. The slides were prepared for cytogenetic analysis according to the methods of Adler [18] and Preston *et al.* [19]. Slides prepared for chromosome aberrations were also used to determine the mitotic index (MI).

Scoring and Statistical Analysis: Fifty well- spread and distinctly identifiable metaphases from each mouse were analyzed. Each selected metaphase was examined using the 100x oil immersion objective of a Zeiss microscope for detecting possible chromosome aberrations. Prior to scoring Uscharin effect on the chromosomes, the slides were covered and coded. To determine the proliferative rate (Mitotic index) 1000 bone marrow cells were analyzed. The mitotic index was determined as the number of dividing cells/ 1000 analyzed cells. Statistical analysis was performed by the ANOVA test using SPSS software for windows, version 11.5 [20].

RESULTS

In the present study, the main types of chromosome aberrations scored were structural aberrations and numerical aberrations. Structural aberrations included breaks, centromeric attenuations and centromeric adhesions.Whereas numerical aberrations included hypoploidy, hyperploidy and polyploidy.

Effect of Different Doses of Uscharin on Chromosomal Aberration: The number of cells with numerical aberrations after 24 h of treatment with Uscharin increased with increasing the dose level as compared with the control. They significantly increased in mice treated with 2.5mg ($p< 0.05$) and 5 mg ($p< 0.01$). Whereas, treatment for 48h the increase was significant at the above two doses at $p< 0.05$ (Table1).

Chromosomal breakage and centromeric attenuations were the main type of structural aberrations at the two investigated times. They increased with increasing the dose, with the rate of centromeric attenuation exceeding chromosomal breakage. The increase in cells with chromosomal breakage was found to be significant ($p< 0.05$) at dose levels of 2.5 and 5 mg. Cells with centromeric attenuations were increased significantly ($p< 0.05$) in animals treated with 1.25 and 2.5mg. Animals treated with 5mg had an increase at $p<0.01$ compared to control (Table1). The number of cells with total structural aberrations in mice treated with the 3 doses of Uscharin for 24h and 48h were significantly increased. Such increases were dose- dependent (Fig. 1). The increase in chromosomal aberrations after treatment for 48 hours was slightly higher than for 24 hours in lower doses. However the difference was not statistically significant.

Table 1: Effect of different doses of Uscharin on the chromosomal aberrations in bone marrow cells of SWR/J male mice after 24 & 48 hours of treatment.

				Cells with structural aberrations											
				Cells with numerical aberrations		Break		Centromeric attenuation		Centric adhesion.		Total		Total aberrant cells	
Duration of treatment	Dose (mg/kg)	No. of animals used	No. of cells examined	No.	Mean ± SE	No.	Mean ± SE	No.	Mean ± SE	No.	Mean ± SE	No.	Mean ± SE	No.	Mean ± SE
	Control	12	600	6	0.50 ± 0.223	2	0.166± 0.166	2	0.166± 0.166	2	0.166± 0.166	6	0.50 ± 0.341	12	1.00 ± 0.516
24 hours	1.25	6	300	6	1.00 ± 0.365	1	0.166± 0.166	15	2.50*± 0.223	1	0.166± 0.166	17	2.83*± 0.210	23	3.83*± 0.333
	2.50	6	300	10	1.66*± 0.210	4	0.66*± 0.210	16	2.66*± 0.210	2	0.33± 0.210	22	3.66**± 0.210	32	5.33**± 0.333
	5.00	6	300	14	2.33**± 0.210	5	0.83*± 0.166	21	3.50**± 0.341	2	0.33± 0.210	28	4.66**± 0.210	42	7.00***± 0.258
48 hours	1.25	6	300	5	0.83 ± 0.307	2	0.33± 0.210	17	2.83*± 0.166	2	0.33± 0.210	21	3.50*± 0.500	26	4.33*± 0.760
	2.50	6	300	11	1.83* ± 0.307	5	0.83*± 0.166	19	0.166± 3.16*	2	0.33± 0.210	26	4.33** ± 0.421	37	6.16**± 0.703
	5.00	6	300	11	1.83* ± 0.401	6	1.0* ± 0.000	20	3.33*± 0.210	2	0.33± 0.210	28	4.66** ± 0.421	39	6.50**± 0.806

* Differences are statistically significant from the control group at p<0.05

** Differences are statistically significant from the control group at p<0.01

*** Differences are statistically significant from the control group at p<0.001

Table 2: Effect of different doses of Uscharin on the mitotic index (MI) in bone marrow cells of SWR/J male mice after 24 & 48 hours of treatment.

Duration of treatment	Dose (mg/kg)	No. of animals used	No. of cells examined	No. of dividing cells	Mitotic index Mean ± SE
	Control	12	12000	552	46.00 ± 0.577
24 hours	1.25	6	6000	214	35.66*± 0.333
	2.50	6	6000	189	31.50** ± 0.341
	5.00	6	6000	185	30.83**± 0.542
48 hours	1.25	6	6000	221	36.83* ± 0.401
	2.50	6	6000	197	32.83** ± 0.401
	5.00	6	6000	194	32.33** ± 0.211

* Differences are statistically significant from the control group at p<0.05

** Differences are statistically significant from the control group at p<0.01

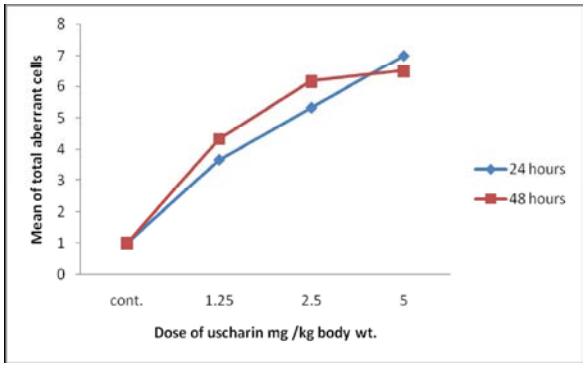


Fig 1: Effect of different doses of Uscharin on mice bone marrow cells with total aberrations (Numerical and structural) after 24 and 48 hours treatment.

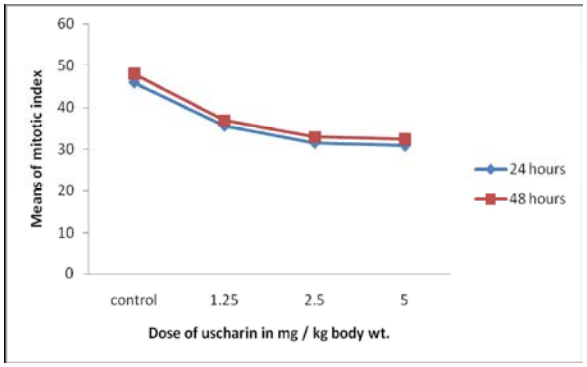


Fig 2: Effect of different doses of Uscharin on mitotic activity of mice bone marrow cells after 24 and 48 hours treatment.

Effect of Different Doses of Uscharin on Mitotic Activity: Uscharin caused a decrease in mitotic activity, as indicated by the mitotic index, in treated mice compared to control ones (Table 2).This decrease in mitotic activity was found to be statistically significant ($p< 0.05$) in mice treated with Uscharin at a dose of 1.25 mg and at $p<0.01$ in mice treated with 2.5 and 5 mg compared to control mice treated for 24h and 48h. The decrease in mitotic activity, after treatment for 48 hours, were slightly higher than for 24 hours. However the difference was not statistically

significant. The decrease in the rate of cell proliferation was found to be dose- dependent after 24 and 48 h of exposure (Fig. 2).

DISCUSSION

Studies of the genetic damage in somatic cells may improve our knowledge about the genotoxic potential of the treatment and also provide an important information for the evaluation of carcinogenic risks [21-24]. Uscharin is currently used as newly designed moluscicide regimes [16, 17], thus, it is important to evaluate its cytogenetic effects.

This study demonstrated that a single dose of Uscharin significantly increased the incidence of cells with numerical aberrations over the control. This increase was statistically significant over the control ($p< 0.05$) in animals treated with 2.5 and 5mg at the two duration times. The ability of Uscharin to induce numerical chromosomal aberrations especially polyploidy is considered an indication for its carcinogenic potential where the relationship between induction of polyploidy by chemical and cancer incidence has been proven [21, 25-27].

Uscharin increased significantly the number of cells with structural aberration over the control and such increase was found to be dose-dependent. Latex-extract from which Uscharin was isolated has been proven to cause cytogenetic effects such as a substantial reduction in mitotic index (MI) and various types of mitotic and meiotic abnormalities in *Vicia faba* L. Such abnormalities were found to be dose and time-dependent [11]. Smit *et al.* [14] and Choedon *et al.* [15] reported that latex-extract induced extensive fragmentation of DNA in Huh-7 (Hepatoma cells) and COS-1 (Non-hepatoma cells) culture cells.

The high incidence and more pronounced type of structural chromosome aberrations reported in this study were centromeric attenuations. Although cells with centromeric attenuations increased with doses, the consequences of such an aberration are not yet confirmed. Centromeric attenuation has been reported as a cause of exposure to mutagenic substances such as synthetic food colour erythrosine [28], nonsteroidal anti-inflammatory drug, Piroxicam [29] and *Lupinus albus* (Termis) and *Hyphaene thebaica* (Doum) [30]. The increase in chromosomal breakage induced by Uscharin which was also found to be dose dependent indicated that Uscharin may have clastogenic potential.

The inhibition of mitotic activity in bone marrow cells reported in this study indicated that Uscharin was cytotoxic. The cytotoxic potential of Uscharin is in accordance with previous studies evaluated cytotoxic and anti-mitotic activities of the dried latex of *C. procera* that significantly inhibited the growth and mitotic activity of *Alium cepa* roots in a dose-dependent manner [12]. It showed antimitotic activity on sea urchin egg development and anti-proliferative activity in mice bearing Sarcoma 180 tumor [13].

In conclusion, the present study demonstrated that Uscharin may have a genotoxic and cytotoxic potentials which is demonstrated by the significant increases in chromosomal aberrations and the decrease in mitotic activity. Further investigation is needed to evaluate the mechanism(s) by which Uscharin induce genetic damage.

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